United States Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

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Dr. Phil Bregitzer
Lead Scientist
Project: 5366-21000-028-00D
Genetic Improvement of Barley and Oats for Enhanced Quality and Biotic Stress Resistance
USDA Agricultural Research Service
1691 S 2700 W
Aberdeen, ID 83210

Re: Regulatory Status of products created by transgene delivery system for barley based on maize Activator/Dissociation (Ac/Ds) transposable elements

Dear Dr. Bregitzer:

Thank you for your letter dated April 15, 2016 inquiring whether barley plants generated using a transgene delivery system based on the maize *Activator/Dissociation (Ac/Ds) transposable element* described in your letter would be regulated articles.

The Plant Protection Act (PPA) of 2000 gives USDA the authority to oversee the detection, control, eradication, suppression, prevention, or retardation of the spread of plant pests or noxious weeds to protect the agriculture, environment, and economy of the United States. The APHIS mission is to protect the health and value of American agriculture and natural resources.

APHIS regulates the importation, interstate movement and environmental release (field testing) of certain genetically engineered (GE) organisms that are, or have the potential to be, plant pests. Regulations for GE organisms that are or have the potential to be plant pests, under the PPA, are codified at 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason To Believe Are Plant Pests." Under the provisions of these regulations, a GE organism is deemed a regulated article if it has been genetically engineered using a donor organism, recipient organism, or vector or vector agent that is listed in §340.2 and meets the definition of a plant pest, or that is an unclassified organism and/or an organism whose classification is unknown, or if the Administrator determines that the GE organism is a plant pest or has reason to believe it is a plant pest.

In your April 15, 2016 letter, you describe your transgene delivery system for barley as one where the final barley lines will contain as yet unspecified inserted DNA, but, will lack any inserted plant pest sequences from the transformation method. Prior to specifying the nature of the GE product, you wish to know whether, in principal, APHIS would consider such organisms

to have nonregulated status. Based on your description of the transformation process and the absence of a specific end product, APHIS provides the following response.

The transgene delivery system described in your letter utilizes *Agrobacterium* to introduce the gene of interest. The gene of interest is cloned into an *Agrobacterium* binary vector that contains plant pest DNA (T-DNA border sequences and the NOS terminator). APHIS must assume the gene of interest, promoters, and terminators may be sourced from plant pests as you have not requested an inquiry for a specific product and you have indicated plant pest may be used. In the transformation, the gene of interest is immediately flanked on both sides by maize Ds elements. In the first step, the binary vector is transformed into barley using *Agrobacterium* mediated transformation. In the second step, transformed barley lines are crossed to a barley line harboring an *AC* transposase gene. In progeny derived from this cross, the transposase results in the transposition of the gene of interest flanked by maize Ds elements into a new genomic region away from the T-DNA vector sequences. The third step involves selecting segregants that lack the plant pest sequences (T-DNA vector/selectable marker, NOS terminator) but retain the gene of interest flanked by maize Ds elements.

In particular:

1. You seek to know the regulatory status of plants at the third step (those lacking any plant pest sequences) if a plant pest was used in the first step.
2. If using a plant pest sequence in the first step causes APHIS to regulate the organisms at the third step, you wish to know whether APHIS would reach the same conclusion if no plant pest sequences and biolistic transformation were used at the first step.
3. Assuming APHIS does not consider barley to be regulated when barley genes of interest are introduced by the Ac/Ds system, you wish to know whether barley transformed with genes from any non-plant pest source would similarly be not regulated.

In the first instance, you correctly acknowledged, any barley transformed with *Agrobacterium* or biolistically transformed with the pCAMBIA 1300 vector would trigger regulation under 7 CFR part 340 because *Agrobacterium* is a plant pest and the pCAMBIA 1300 vector contains plant pest sequences. Thus, under the system you describe in your letter, APHIS would consider the engineered barley to be regulated in the first step and would evaluate the barley on a case by case basis in the third step.

In the second instance, as long as no plant pests were used as donors for the gene of interest or its promoters, terminators, or other associated genetic elements, APHIS would not consider plants generated in either the first step in the transformation process or the third step to be regulated. If plant pests were donors for the gene of interest or its associated genetic elements, APHIS would consider plants generated in both the first and third steps to be regulated. If you have any

concerns about a product's regulatory status in this instance, we would encourage you to submit an AIR inquiry to ascertain whether a particular product would be subject to 7 CFR part 340.

In the third instance, our response depends on whether *Agrobacterium* or other plant pest sequences are used to create the GE barley. In past Am I Regulated (AIR) decisions, APHIS has concluded that plant null segregant lines or plant lines modified only by targeted deletion are not subject to 7 CFR part 340. APHIS would again encourage you to submit an AIR inquiry for the specific product if you have any concerns whether it would be regulated under 7 CFR part 340.

In summary, under our regulations it makes a difference whether *Agrobacterium* and or plant pest sequences are used in engineering the barley with regard to regulation under 7 CFR part 340. If no plant pest sequences were used in the first step (i.e. biolistic transformation, and no plant pest sequences in the vector), and if the inserted gene and its associated genetic elements are not from a plant pest, the resulting plants would not trigger the regulations under 7 CFR part 340. If plant pests were used at the first step, we will require evidence that all plant pest sequences have been segregated away from the insert before we can render a decision on the regulated status of the resulting plants.

When APHIS receives AIR inquiries for engineered plants made with or without plant pest sequences, APHIS reviews the request to address whether the engineered plant poses a noxious weed risk. APHIS would consider regulating the plant under the noxious weed regulation, 7 CFR part 360, if it were determined to pose a noxious weed risk; APHIS has the option to regulate the plant under 7 CFR part 360 regardless of whether or not it was engineered using plant pest sequences. APHIS has reviewed the available scientific literature and has concluded that cultivated barley (*Hordeum vulgare*) itself is not a noxious weed nor is it listed as a noxious or invasive weed in any state or county. However, APHIS is aware there are several weedy or invasive barley species in the United States, distributed from the Dakotas to California, e.g., *H. jubatum* (foxtail barley), *H. marinum* ssp. *gussoniamum* (Mediterranean barley), and *H. marinum* ssp. *leporinum* (hare barley). Several of the wild species, including *H. jubatum, H. Mediteranean* ssp. *gussoniamum, and H. marinum* ssp. *leporinum,* have different chromosome numbers reducing the potential for crosses. Because of this, and other differences gene flow from cultivated barley to wild relatives resulting in viable progeny has a very low probability.

For example, when forced crosses for two different ploidy species were studied in the laboratory using tissue/embryo culture and subsequently grown out, the resultant progeny either exhibited aneuploidy or low pollen viability (less than 5%) or both[1,2,3]. Such results indicate a cross in the wild would be an extremely rare event.

Please be advised that the importation of barley will be subject to APHIS Plant Protection and Quarantine (PPQ), permit and/or quarantine requirements. For further information, on the importation of barley, you may contact Shailaja Rabindran at 301-851-2167 or contact PPQ general number for such inquiries at (877) 770-5990.

Please be advised that your engineered barley may still be subject to other regulatory authorities such as FDA or EPA.

Should you become aware at any time of any issues or additional information that may affect the Agency's conclusion regarding this inquiry; you must immediately notify the Agency in writing of the nature of the issue. We hope you appreciate our commitment to plant health and support for the responsible stewardship for the introduction of GE plants.

Sincerely,

M. J. Firko

Michael J. Firko, Ph.D.
APHIS Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

3/20/2016

Date

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[1] Bothmer R and Jacobsen N. 1986. Interspecific crosses in Hordeum (Poaceae). Plant systematics and evolution 153, pp. 49-64.

[2] Bothmer RV, Flink J, Jacobsen N, Kotimaki M, and Landstrom T. 1983. Interspecific hybridization with cultivated barley (Hordeum vulgare L.). Hereditas 99, pp. 219-244.

[3] Orton TJ. 1979. A quantitative analysis of growth and regeneration from tissue cultures of Hordeum vulgare, H. jubatum and their interspecific hybrid. Environmental and Experimental Botany 19, pp. 319-335.